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Coordinate augmentation in expression of genes encoding transcription factors and liver secretory proteins in hypo-oncotic states

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Coordinate augmentation in the expression of genes encoding transcription factors and liver secretory proteins in hypo-oncotic states.

Background. In the nephrotic syndrome (NS) proteins of intermediate size (40 to 200 kD) are lost into the urine resulting in a decrease in plasma albumin concentration and as a consequence a reduction in plasma colloid osmotic pressure (π). Plasma π has also been reported to be reduced in the condition of hereditary analbuminemia. The liver, in an apparent compensatory response, increases synthesis of a group of secreted proteins defending plasma π. Regulation of several of these proteins, including both positive and negative acute phase proteins, is at the transcriptional level. This is the only known condition in which transcription of both positive and negative acute phase proteins (APPs) are increased simultaneously. The specific transcription factor(s) that might regulate this cascade is not defined.

Methods. RNA was extracted from livers of 5 rats with hereditary analbuminemia (the Nagase analbuminemic rat, NAR), 5 rats with NS induced by adriamycin (Adria), 5 rats with NS caused by passive Heymann nephritis (NS) and 5 control animals. The concentrations of mRNAs encoding four secreted proteins (albumin, transferrin, fibrinogen, and apo A-1), five transcription factors, early growth response factor 1 (EGRF–1), HNF-4, NGFI-C, EGR-3, and Krox20 relative to two housekeeping genes, β actin and GAPDH were determined simultaneously using kinetic reverse transcriptase polymerase chain methodology (kRT-PCR).

Results. The levels of all mRNAs encoding secreted proteins except for albumin (which was reduced in NAR) were increased in NS and NAR and correlated significantly with one another. mRNA encoding EGRF 1 was increased fivefold in NS and NAR, and correlated significantly with mRNAs encoding Apo A-1, transferrin and albumin in the two NS groups. HNF-4 mRNA was increased approximately twofold in both NS groups and correlated with albumin ($R = 0.881$, $P < 0.001$), transferrin ($R = 0.563$, $P = 0.012$) and apo A-1 ($R = 0.644$, $P = 0.003$). While fibrinogen mRNA correlated with that of each of the other secreted proteins, it did not correlate with either HNF-4 or EGRF-1 mRNA. Krox20, EGR3 and NGF1C were expressed at nearly undetectable levels.

Conclusions. The hepatic response in conditions characterized by reduced plasma π include increased levels of mRNAs encoding a group of secreted proteins, including the negative APPs albumin, transferrin and apo A-1, and the positive APP fibrinogen. Levels of mRNAs encoding negative APPs and fibrinogen correlate with one another, suggesting that they are coordinately controlled. Both EGRF-1 and HNF-4 may regulate the expression of the negative APPs, which have increased transcription in hypo-oncotic states.

The nephrotic syndrome is characterized by the loss into the urine of proteins of intermediate sized proteins with a resultant decrease in plasma colloid osmotic pressure (π) [1]. Synthesis of a group of proteins secreted by the liver is increased in the nephrotic syndrome, resulting in a normal or near normal plasma protein concentration [2]. Synthesis of several of these proteins is regulated transcriptionally [3–7]. Rats with hereditary analbuminemia also have reduced plasma π because of a failure to synthesize albumin as a consequence of a deletion in the MN intron leading to a splicing error [8], do not have albuminuria, but also increase the levels of a variety of liver-derived proteins in serum. As in the nephrotic-derived syndrome, albumin gene transcription is actually increased, but intact message does not leave the nucleus [9]. Synthesis of the same proteins secreted by the liver that are found to be increased in the nephrotic syndrome is increased in hereditary analbuminemia as well. As in the nephrotic syndrome, this also is regulated at the level of transcription for proteins, including apolipoprotein A-1 (apo A-1) [5], transferrin [6] and fibrinogen [7].

The rate of synthesis of several liver secreted proteins correlates with one another, raising the possibility that all of these proteins are responding to the same stimulus,

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such as plasma π. Such a hypothesis suggests that the same signal transduction mechanisms would likely be utilized and that this process might act through the same set of *trans*-acting transcription factors. Recently, Zaiou et al found that mRNA encoding the leucine zipper transcription factor early growth response factor 1 (EGRF–1) contributed to increased expression of apo A-1 in mice with an experimental model of the nephrotic syndrome [10]. While the level of this transcription factor increased approximately fivefold, the level of another liver specific factor that binds to the apo A-1 promoter, HNF-4, did not. Combined with reduced induction of hepatic apo A-1 in EGRF-1 knockout mice, these results were interpreted as indicating that "basal and induced apo A-1 gene expression *in vivo* are regulated by the *trans*-acting factor EGRF-1 and require the same *cis*-acting elements in the core promoter." These observations suggested to us that the same transcription factors might be responsible for induction of secreted protein transcripts in nephrotic rat liver and that regulation of transcription factors might provide insights into the mechanism of regulation of hepatic gene expression by plasma π.

First, we sought to verify that EGRF-1 (also known as NGF1A) mRNA was induced (relative to GAPDH and β-actin) in rat liver as had been observed for mouse. Second, we sought to establish the relationship between expression of mRNAs encoding several proteins secreted by the liver and mRNAs encoding transcription factors that might regulate their expression.

Early growth response factor-1 belongs to a family of zinc finger proteins with shared consensus DNA binding specificity that are involved in immediate-early growth response [11]. If these factors were expressed in rat liver, it seemed possible that expression of other family members (NGFI-C, EGR-3, or Krox20) might play roles in induction of transcription of a cohort of proteins secreted by the liver in the nephrotic syndrome. HNF-4 plays a role in regulating the expression of a group of proteins that are changed coordinately with nutritional status [12], making it a good candidate for causing up-regulation of the same group of secreted proteins. Since kinetic RT-PCR provides a very sensitive as well as quantitative assay, the expression of these latter transcripts was also examined.

METHODS

Experimental animals

Fifteen male Sprague Dawley rats 150 to 180 g (Simenson Farms, Hayward, CA, USA) were kept in temperature regulated, standard 12 hour light/dark cycled rooms. Two models of the nephrotic syndrome were used so as to avoid model-induced bias. In one, passive Heymann nephritis was induced by intraperitoneal injection of rabbit Anti-FX IA antiserum (Dr. Florence Hutchison, Charleston, SC, USA). In the other adriamycin (5 mg/kg Adriamycin RDF; Pharmacia & Upjohn, Milan, Italy) was injected into a tail vein in a dose of 5 mg/kg. Animals were placed in a metabolic cage two weeks after injection of anti-FX IA serum or four weeks after injection of adriamycin to establish whether or not proteinuria was present. To obtain livers, rats were then anesthetized with sodium pentobarbital (30 mg/kg) by intraperitoneal administration. The abdomen was incised and the liver perfused retrograde with iced saline. The liver was then removed, weighed and RNA was extracted as described subsequently.

Rat liver total RNA was isolated by homogenization in guanidinium isothiocyanate/Na acetate/mercaptoethanol using the method of Srivastava, Srivastava and Schonfeld [13]. The RNA samples were treated with DNAse I to hydrolyze residual contaminating DNA. DNAse I treatment was performed for 30 minutes at 37°C with 4 U (Promega) per 100 μg RNA in a buffer containing 10 mM Tris acetate pH 7.5, 10 mM magnesium acetate, 50 mM potassium acetate, and 36 U of RNase inhibitor (Pharmacia). Following digestion, NaCl was added to 150 mM and samples were extracted with phenol that had been saturated with NNES (50 mM sodium acetate pH 5.0, 100 mM NaCl, 10 mM EDTA, 0.5% SDS). The resulting aqueous phase was extracted with chloroform. Residual organic solvents and salt were removed by two ethanol precipitations. First, RNA was precipitated with sodium acetate, added to 0.2 M and 2.5 volumes of 95% ethanol. The pellet was washed with ethanol and vacuum dried. RNA was resuspended in TE (10 mM Tris pH 7.0, 1 mM EDTA) for a second precipitation from 2 M ammonium acetate using 2.5 volumes of 95% ethanol. After vacuum drying (SpeedVac), RNA pellets were resuspended in 100 μl TE and concentration was determined by A260. For all 20 samples, at least 85% of the starting material was recovered with A260/A280 ratios around 2.0, consistent with high quality RNA. The resulting RNA preparations were free of detectable genomic DNA contamination since they did not serve as template for PCR amplification by *Taq* or *Tth* DNA polymerase without a reverse transcriptase step after 50 PCR cycles.

Kinetic RT-PCR instrumentation

The kinetic thermal cycler (KTC) used in the experiments described below is built on a standard Perkin Elmer 9600 thermal cycler platform [14]. The reaction block (96 or 384 reactions) is illuminated with a UV light source mounted directly above the reaction block. Incident UV light passes through a dichroic mirror mounted at 45° to the reaction block. Ethidium bromide fluorescence is reflected by the dichroic mirror to an 8 bit CCD camera mounted at 90° to the reaction block. During the annealing phase at each cycle of the polymer-

ase chain reaction, a shutter opens transiently to expose the reaction block to full field UV illumination while the 8 bit digital (CCD) camera takes two exposures. The exposures are transmitted via video grabber board and stored on a PC compatible microcomputer as TIF images. The KTC software averages two and integrates light values for a predetermined area over each reaction well in the array. For each polymerase chain reaction (PCR) cycle, the average integrated light values for each reaction well are stored in spreadsheet format, providing the raw data for analysis. Thus, for a typical 50 cycle run, each reaction generates 50 kinetic data points.

Kinetic RT-PCR reaction conditions

Composition of the kRT-PCR reaction assay. The kRT-PCR reaction incorporated a number of important features in a single reaction. Reactions were performed with or without capless tubes with a transparent oil overlay. Each reaction (10 ml) included the following components: 50 mM Tricine buffer; pH 8.3, 110 mM potassium acetate; 13% glycerol; 0.3 mM dATP, dCTP, and dGTP; 0.05 mM dTTP; 0.5 mM dUTP; 2.4 mM Mn $(OAc)_2$; 2.5 mM ethidium bromide; 0.25 mM primers; 1 unit r*Tth* DNA polymerase; 0.2 units UNG; and the indicated amount of total cellular RNA template. To prevent interference by product carryover, all PCR reactions are performed with a nucleotide mixture which contains a molar excess of dUTP over dTTP. Kinetic RT-PCR is performed in three phases. In the first phase, a two minute incubation at 50°C, allows the thermostable uracil N-glycosylase (UNG) to hydrolyze any dU-containing PCR product carryover.

The second phase of the reaction is the reverse transcriptase (RT) step (30 min at 60°C). Reaction conditions (in particular; buffer, nucleotide concentration, temperature, and Mn acetate concentration) have been optimized for the reverse transcriptase reaction. At 60°C, UNG activity is severely reduced and the *Tth* DNA polymerase exhibits optimal reverse transcriptase activity. Thermostable *Tth* DNA polymerase is used in these reactions because this enzyme exhibits high levels of RNA-dependent and DNA-dependent DNA polymerase activity. The reaction buffer has been formulated to provide the broadest Mn^{2+} optimum for the reverse transcriptase activity.

The final phase of the reaction is DNA amplification by thermal cycling during which kinetic data is collected. Once the DNA template has been created by reverse transcription, DNA dependent DNA polymerase-dependent product accumulation occurs as in conventional PCR. The inclusion of ethidium bromide in the reaction permits fluorescent detection of double-stranded DNA product as it accumulates with each thermal cycle. It is important to emphasize that the reverse transcriptase step and the PCR amplification steps are performed in the same reaction by the same thermostable DNA polymerase.

Primer design and validation. For each transcription factor gene, at least two primer pairs that were expected to specifically amplify the target transcript were designed, synthesized, and tested as previously described [14]. As expected for gene specific amplifications, independent primer pairs directed at the same target transcript produced similar C_t's in the kRT-PCR assay. One primer pair was selected for further study of each target transcript. None of the primer pairs used produced C_t's for template independent products (primer dimer) at cycles earlier than 39, demonstrating that these primer pairs had very low potential to form primer dimer.

Transcript level determinations. Kinetic RT-PCR was performed in 100 μl reactions as described [14]. All assays were performed at least in duplicate with livers isolated from five different animals of each type. Potential binding sites for transcription factors were identified using the TFBIND program (http://tfbind.ims.u-tokyo.ac.jp/) to analyze flanking sequences upstream of the transcription start site [15].

Data analysis. C_t values were measured at an arbitrary fluorescence level of 1.5. Data were computed relative to GAPDH or to β-actin as an internal standard.

Statistical analysis

Groups were compared by one-way analysis of variance. Linear regression analysis was performed by the method of least squares. Normally distributed data were analyzed by Dunnett's method. Data that were non-normally distributed were analyzed using multiple comparisons vs. control group by Dunn's method.

RESULTS

Secreted proteins

Apolipoprotein A-1, transferrin, and fibrinogen mRNAs were all significantly increased in all of the experimental groups compared to controls (Fig. 1), and albumin mRNA was significantly increased in both nephrotic groups. Albumin mRNA was significantly decreased in NAR (Fig. 1); however, the level of this mRNA is unlikely to correlate with its rate of transcription since the gene is transcribed but the RNA degraded prior to transport from the nucleus. We therefore did not include albumin data from this group in regression analysis of the relationship between transcription factors and albumin mRNA.

Albumin mRNA correlated with the three other secreted proteins (Apo A-1, fibrinogen and transferrin) (Fig. 2). Apo A-1 also correlated with both fibrinogen and transferrin (Fig. 3), and fibrinogen and transferrin also correlated with one another (Fig. 4), which are compatible both with our previous observations in individual

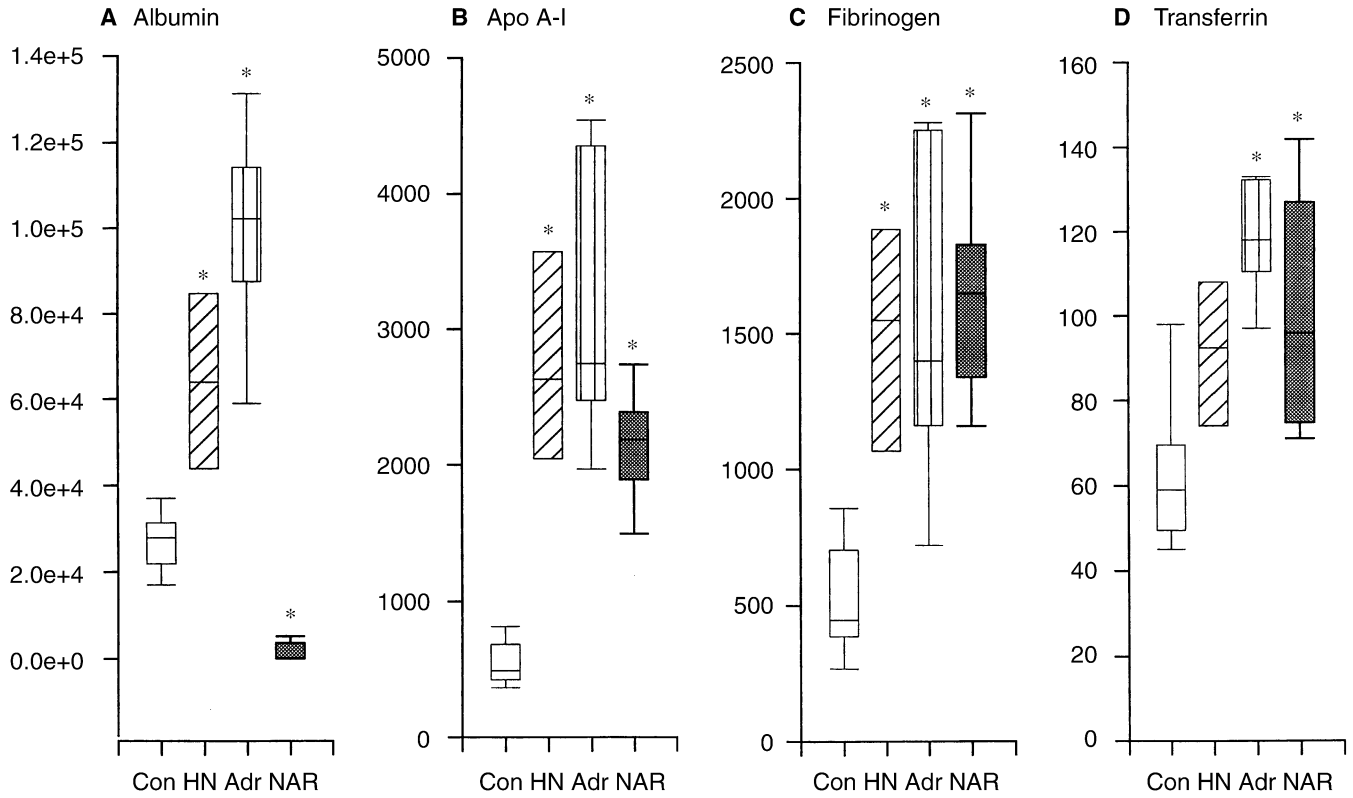

Fig. 1. (*A*) Level of mRNA encoding albumin relative to glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA in livers from five control rats (Con), four rats with passive Heymann nephritis (HN), five rats with adriamycin-induced nephrotic syndrome (Adr) and five Nagase analbuminemic rats (NAR). (*B*) Level of mRNA encoding apolipoprotein A-1 (apo A-1) relative to GAPDH as in panel A. (*C*) Level of mRNA encoding fibrinogen relative to GAPDH as in panel A. (*D*) Level of mRNA encoding transferrin relative to GAPDH as in panel A. *$P < 0.05$ vs. Con.

models of reduced plasma π [5–7] and suggest that all might be regulated by the same group of transcription factors under these conditions.

Albumin mRNA correlated closely with HNF-4 (R = 0.881, $P < 0.001$) and less well with EGRF-1 (R = 0.561, $P = 0.037$) (Fig. 5). Apo A-1, like albumin, correlated best with HNF-4 (R = 0.622, $P = 0.004$) and less well with EGRF-1 (R = 0.428, $P = 0.067$) (Fig. 6). Despite this correlation, HNF-4 mRNA was not increased in NAR (Table 2) while apo A-1 mRNA was increased (Fig. 1).

While fibrinogen β chain was also increased significantly in all three experimental groups (Fig. 1), the level of this mRNA did not correlate with either transcription factor. Fibrinogen mRNA, however, did correlate both with that of albumin ($r = 0.809$, $P < 0.001$) (Fig. 2) and with apo A-1 ($r = 0.866$, $P < 0.001$) (Fig. 3). This correlation remained highly significant even when the control group was eliminated from the relationship (R = 0.701, $P < 0.005$).

Transferrin mRNA was significantly increased both in NAR and Adria (Fig. 1). Transferrin mRNA also tended to be increased in HN compared to control but this difference failed to achieve significance. As in the case of the other secreted proteins, transferrin mRNA correlated positively both with HNF-4 (R = 0.563, $P = 0.012$) and with EGRF-1 (R = 0.476, $P = 0.04$; data not shown). Transferrin mRNA also correlated positively with the other secreted proteins, albumin mRNA ($r = 0.759$, $P = 0.002$), apo A-1 (R = 0.612, $P = 0.005$), and fibrinogen (R = 0.522, $P = 0.022$).

Transcription factors

Early growth response factor–1 was increased in NAR, HN and Adria when GAPDH (Table 1A) or when β-actin was used as the normalizing mRNA (Table 1B). Although HNF-1 correlated much more closely with the levels of mRNA's encoding secreted proteins, the level of this transcription factor was only increased about two-fold in both nephrotic groups regardless of whether GAPDH (Table 2A) or β actin (Table 2B) was used for normalization and was not increased in NAR.

The three remaining related transcription factors (Krox20, EGR3, and NGF1C) were not present in liver in physiologically significant levels. Each of these were expressed at a level that was more than 100-fold lower than EGRF-1.

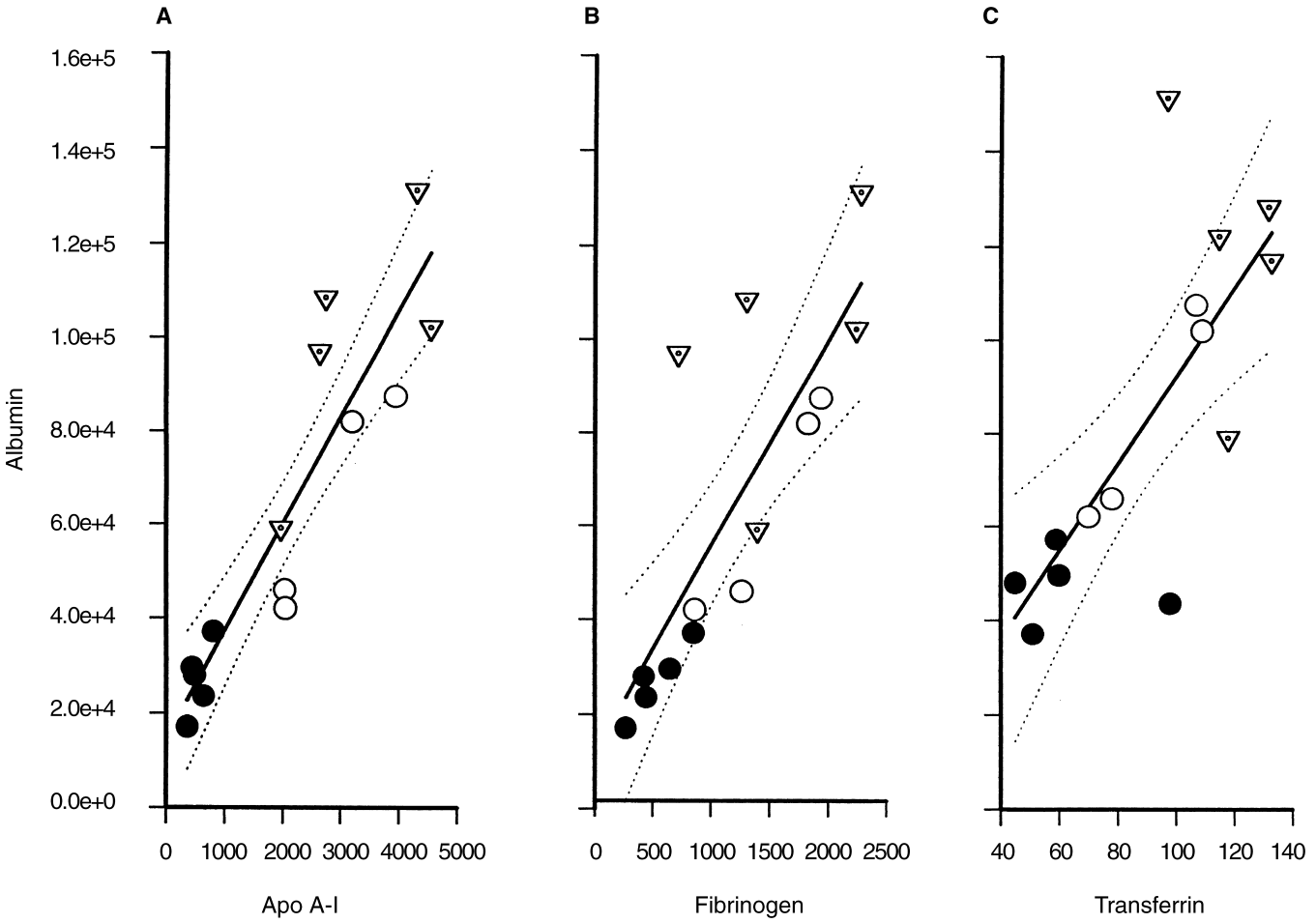

Fig. 2. (*A*) Relationship between albumin mRNA (relative to GAPDH) and apo A-1 mRNA (relative to GAPDH) in control rats (closed circles; Con), rats with Heymann nephritis (open circles; HN) and rats with adriamycin-induced nephrotic syndrome (inverted dotted triangles; Adr); $r = 0.908$, $P < 0.001$. (*B*) Relationship between albumin and fibrinogen mRNA as in panel A; $r = 0.809$; $P < 0.001$. (*C*) Relationship between albumin and transferrin mRNA as in panel A; $r = 0.759$, $P = 0.002$.

Putative interaction with flanking sequences

TFBIND identified seven putative sites for HNF-4 binding and six putative sites for EGRF-1 binding in the 5′ flanking sequences of the rat apolipoprotein A-1 gene. Examination of the upstream sequences of the rat fibrinogen β gene revealed eight and four putative binding sites for HNF-4 and EGRF-1, respectively. Upstream gene sequences were not available for rat albumin or transferrin. However, TFBIND identified at least seven potential binding sites for either HNF-4 or EGRF-1 in the mouse albumin and transferrin 5′ regions.

DISCUSSION

The nephrotic syndrome is characterized by urinary protein loss and an alteration in plasma protein composition that results both from the loss of intermediate sized proteins into the urine and augmentation specifically of synthesis of a group of proteins secreted by the liver. These include both positive (fibrinogen, α2-macroglobulin) [7, 16–18] and negative acute phase proteins (albumin, transferrin, apo A-1) [2–6]. We have previously established that increased synthesis of four of these proteins is regulated at the transcriptional level [3, 5–7, 19]. The alteration in protein synthesis observed in the nephrotic syndrome in the rat is mirrored by similar changes in nephrotic patients [16, 18].

HNF-4 is one of several transcription factors that plays a role in altering expression of a group of genes encoding proteins synthesized in the liver that respond to nutritional status [12, 20] including transferrin [21] and apo A-1 [22–24], and apo C III [25], acting as an enhancer of transcription. The DNA binding activity of HNF-4 may also be modified during dietary protein restriction [12], suggesting that both the levels of this protein as well as post-translational alteration in its structure may play a role in its regulatory repertoire. Thus, the increased levels of apo A-1 mRNA observed in our study

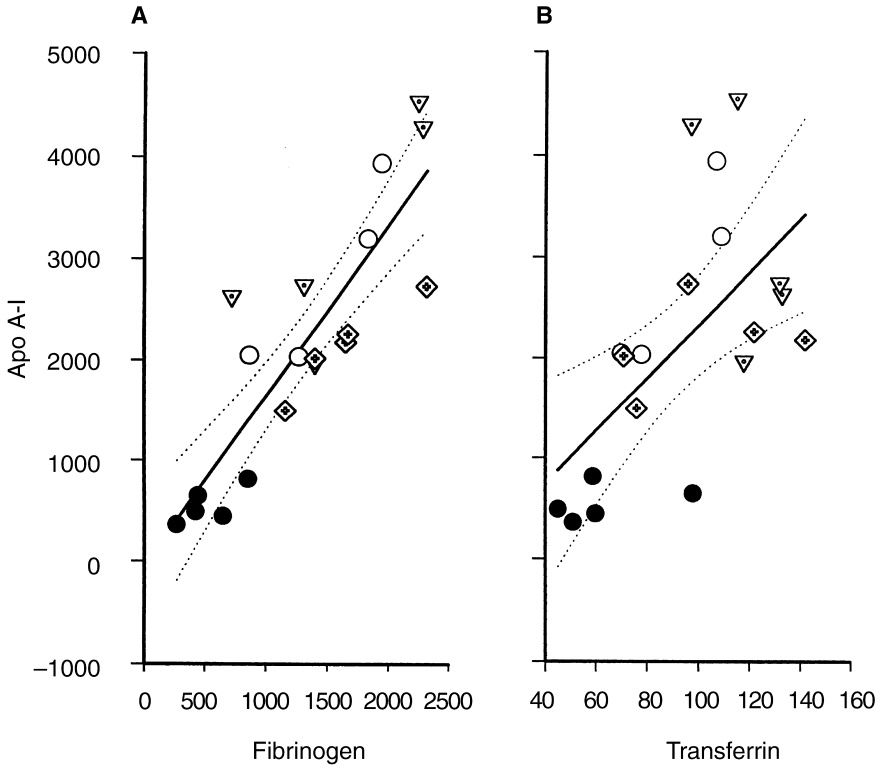

Fig. 3. (*A*) Relationship between apo A-1 mRNA (relative to GAPDH) and fibrinogen mRNA (relative to GAPDH) in control rats (closed circles), rats with Heymann nephritis (open circles), rats with adriamycin induced nephrotic syndrome (dotted inverted triangles), and Nagase analbuminemic rats (cross-centered diamonds; NAR); $r = 0.901$, $P < 0.01$. (*B*) Relationship between apo A-1 and transferrin mRNA. Symbols as in (*A*); $r = 0.612$, $P = 0.005$.

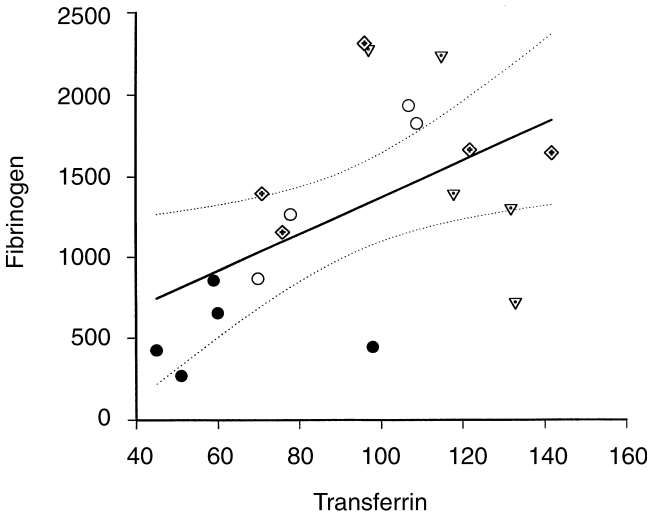

Fig. 4. Relationship between fibrinogen (relative to GAPDH) and transferrin mRNA. Symbols are: control rats (closed circles); rats with Heymann nephritis (open circles); rats with adriamycin induced nephrotic syndrome (inverted triangles); Nagase analbuminemic rats (cross-centered diamonds; NAR). $r = 0.522$; $P = 0.022$.

in one experimental group (NAR), presumably resulting at least in part from increased transcription of the cognate gene, does not exclude a regulatory role of HNF-4 in this process since it has been demonstrated to act by means other than increased expression of its gene alone.

Zaiou et al found in a transgenic mouse model that the *trans*-acting factor EGRF-1 most likely played a role in augmentation of apo A-1 transcription [10]. They found no role for HNF-4, however, in the regulation of apo A-1 transcription. The change in EGRF-1 mRNA levels between groups and within each group has the greatest variability and is known to bind to the 5′ flanking sequence of apo A-1, thus making it a likely candidate for a transcription factor involved in regulation not only of apo A-1, but of all the negative acute phase proteins whose rate of transcription is increased in the nephrotic syndrome as well as in analbuminemia.

Our observations are consistent with theirs regarding a potential relationship between EGRF-1 and alteration in apo A-1 gene expression; however, we also find that HNF-4 is increased approximately twofold in both models of the nephrotic syndrome. Also, HNF-4 correlates more strongly with both albumin and apo A-1 and the other secreted negative acute phase proteins than does EGRF-1. Taken in the context of work by others suggesting that HNF-4 plays a role in regulating transcription of a group of genes encoding a number of liver derived proteins [12, 16–18, 21], our data suggest that HNF-4 may also be an important transcription factor that is responsible for the alteration in synthesis of this group of proteins secreted by the liver when plasma π is reduced.

In contrast to what occurs in the two models of the nephrotic syndrome, HNF-4 was not increased in NAR

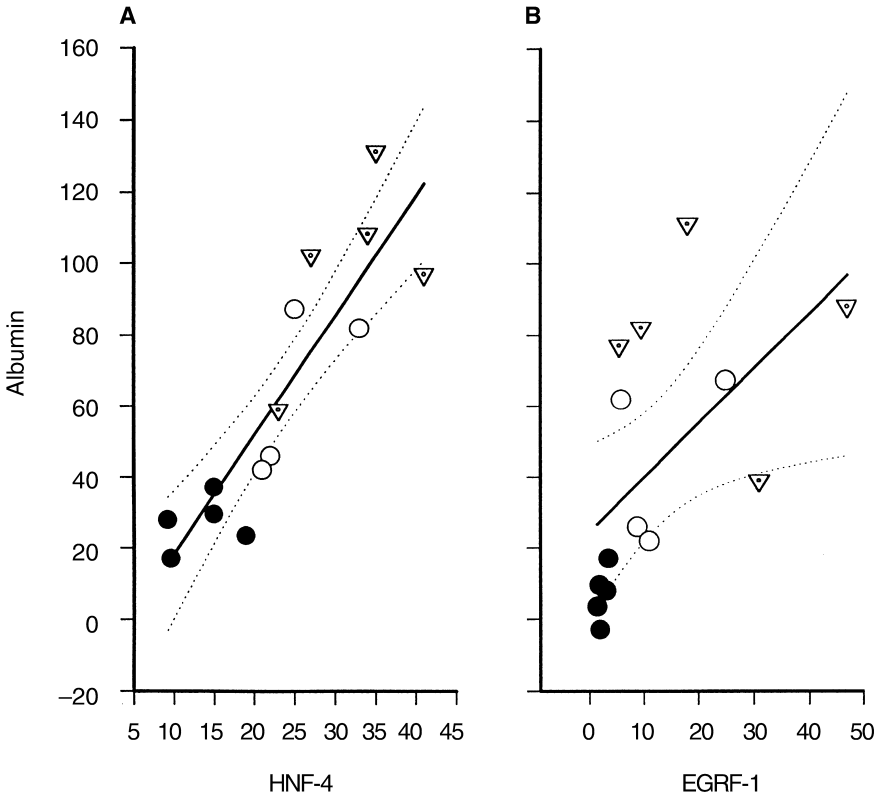

Fig. 5. (*A*) Relationship between albumin mRNA (relative to GAPDH) and hepatocyte nuclear factor 4 mRNA (HNF-4) (relative to GAPDH). Symbols are: control rats (closed circles); rats with Heymann nephritis (open circles); rats with adriamycin induced nephrotic syndrome (dotted inverted triangles). $r = 0.881$; $P < 0.001$. (*B*) Relationship between albumin mRNA and early growth response factor 1 (EGRF-1) mRNA (relative to GAPDH). $r = 0.5598$; $P = 0.037$.

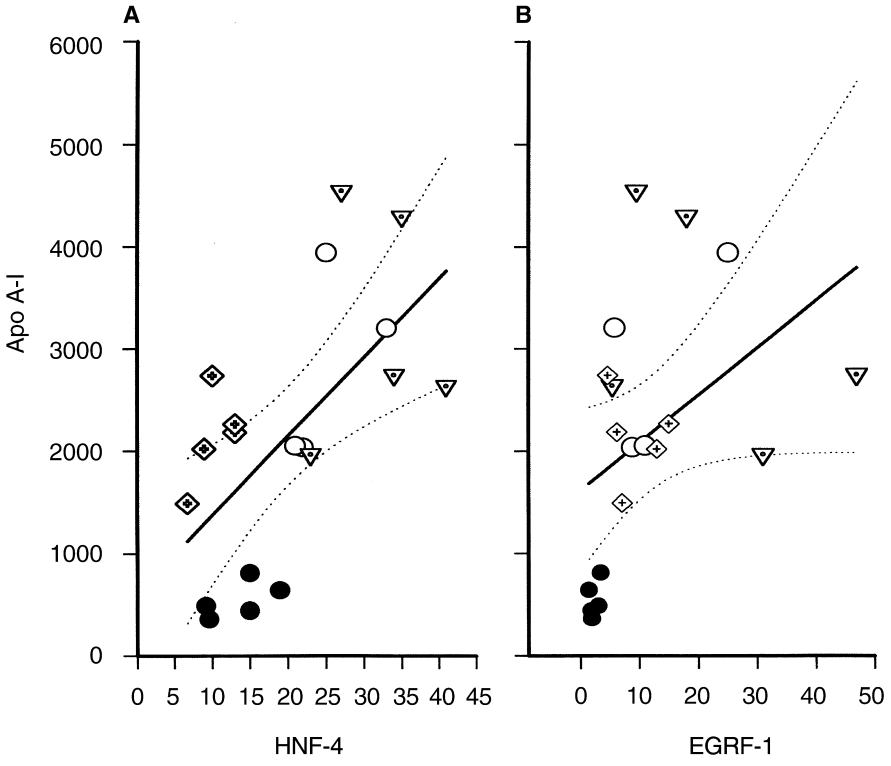

Fig. 6. (*A*) Relationship between apo A-1 mRNA (relative to GAPDH) and HNF-4 mRNA. Symbols are: control rats (closed circles); rats with Heymann nephritis (open circles); rats with adriamycin induced nephrotic syndrome (dotted inverted triangles); Nagase analbuminemic rats (cross-centered diamonds; NAR). $r = 0.622$; $P = 0.004$. (*B*) Relationship between apo A-1 mRNA and EGRF-1 mRNA. $r = 0.428$; $P = 0.067$.

Table 1A. Concentration of EGRF-1 mRNA in rat liver

Group name	*N*	Mean ± SD	Ratio/control	*P*
Control	5	2.3 ± 0.85	1	
HN	4	12.7 ± 8.5	5.5	
Adria	5	22.2 ± 17.0	9.64	< 0.05
NAR	5	9.2 ± 4.5	3.99	

Differences are vs. Control using one way ANOVA by Dunnett's method. Concentrations of early growth response factor 1 (EGRF-1) mRNA are relative to GAPDH mRNA in normal Sprague Dawley rats (Control), rats with passive Heyman nephritis (HN), adriamycin-induced nephrotic syndrome (Adria) and Nagase analbuminemic rats (NAR).

Table 1B. Concentration of EGRF-1 mRNA in rat liver

Group name	*N*	Median	25%	75%	Ratio/control	*P*
Control	5	0.66	0.328	0.79	1	
HN	4	2.225	1.54	5.155	3.37	< 0.05
Adria	5	2.84	1.773	4.902	4.30	< 0.05
NAR	5	2.07	1.865	3.497	3.14	

Concentrations of EGRF 1 mRNA are relative to β-actin mRNA in Control, HN, Adria and NAR. Statistics are vs. Control. These data were not normally distributed and were therefore anallyzed using Multiple Comparisons versus Control Group by Dunn's method.

Table 2A. Concentration of HNF-4 mRNA in rat liver

Group name	*N*	Mean ± SD	Ratio/control	*P*
Control	5	13.6 ± 4.1	1	
HN	4	25.3 ± 5.4	1.86	0.043
Adria	5	32 ± 7.0	2.36	0.013
NAR	5	10.3 ± 2.7	0.76	

Concentration of hepatocyte nuclear factor 1 (HNF-1) mRNA relative to GAPDH mRNA in Control, HN, Adria and NAR vs. Control. Multiple comparisons were made versus the Control group using Dunnett's method.

Table 2B. Concentration of HNF-4 mRNA in rat liver

Group name	*N*	Mean ± SD	Ratio/control	*P*
Control	5	3.62 ± 0.72	1	
HN	4	5.85 ± 1.55	1.79	< 0.05
Adria	5	6.2 ± 1.81	1.90	< 0.05
NAR	5	3.12 ± 0.88	0.96	

Concentration of HNF-1 mRNA relative to β-actin mRNA in Control, HN, Adria and NAR. Statistics are vs. Control using Dunnett's method.

while mRNAs encoding apo A-1, fibrinogen, transferrin and EGRF-1 are increased. This pattern of regulation is similar to what has been described in the mouse. Increases in the levels of EGRF-1 mRNA alone (NAR) or in combination with HNF-4 (nephrotic models) could mediate the observed effects on secreted proteins.

Examination of the flanking sequences of the genes encoding all of the secreted proteins reveals that there is clearly opportunity for these transcription factors to regulate their transcription. TFBIND identified one HNF-4 and six EGRF-1 potential binding sites upstream of the rat EGRF-1 gene, raising the possibility that EGRF-1 transcription may be autoregulated or that there may be regulation of EGRF-1 by HNF-4.

Neither of these transcription factors correlated with fibrinogen, yet fibrinogen mRNA correlated closely with albumin and Apo A-1. This observation is similar to our previous reports using a different method to measure RNA (Northern blot), and suggests that regulation of all of these transcriptionally regulated proteins may be coordinately controlled in hypo-oncotic states in the rat. The lack of correlation between fibrinogen mRNA and that of either transcription factor may be due to sample size and represent a type II error. Alternatively, it is possible that while both EGRF-1 and HNF-4 play a role in regulating expression of the three negative acute phase proteins, they do not participate in regulation of fibrinogen gene expression, and while the concentration of fibrinogen mRNA correlates with each of the other secreted proteins, it may be regulated by different DNA binding proteins. We have excluded the possibility that Krox20, EGR3 or NGF1C is expressed in liver.

The correlation among the RNAs encoding the four secreted proteins whose rate of synthesis is increased in hypo-oncotic states suggests that they may be controlled by similar mechanisms (reduced π). The correlation between each of these and transcription factors known to regulate the expression of at least two of these proteins (transferrin and apo A-1) suggests that these transcription factors may be a component of the repertoire of events that occur in response to decreased plasma π (or albumin concentration) and eventually lend insight into understanding the mechanisms of homeostatic hepatic response to alterations in plasma protein composition.

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